AVIAN DISEASES 54:834–840, 2010

Observations on the Gross Pathology of *Eimeria praecox* Infections in Chickens

P. C. Allen and M. C. Jenkins[A]

Animal Parasitic Diseases Laboratory, Agricultural Research Service, U.S. Department of Agriculture, Building 1040, Room 103, BARC-East, Beltsville, MD 20705

Received 16 October 2009; Accepted and published ahead of print 9 December 2009

SUMMARY. Challenge infections with 10^3, 5×10^4, 10^5, or 5×10^5 sporulated *Eimeria praecox* oocysts caused moderate but significant weight gain reduction at all infective doses. Substantial reduction in plasma carotenoids and moderate but significant increases in plasma $NO_2^- + NO_3^-$ were observed only at the two higher doses when measured at day 6 postchallenge (PC). Daily monitoring of chickens after challenge with 5×10^4 oocysts revealed an inflammatory response in the duodenum and jejunum beginning at day 1 PC that was associated with a significant increase in levels of plasma $NO_2^- + NO_3^-$, which peaked at day 4 PC. A moderate, uniform hyperplasia of the small intestine and significant depression of plasma carotenoids were observed on days 4–6 PC. Plasma $NO_2^- + NO_3^-$ decreased to control levels by day 6 PC. All infections were accompanied by production of a mucoid exudate in the duodenum and jejunum, which became thick and opaque by 4 days PC and tended to obscure mildly inflamed areas. These observations indicate that the acute host response to primary infection with *E. praecox* is both different from and occurs earlier than the response to experimental infections with other *Eimeria* spp., such as *Eimeria acervulina*, *Eimeria maxima*, or *Eimeria tenella*. These factors need to be considered in observations of pathology arising from co-infections of *E. praecox* with other *Eimeria* species, especially in drug sensitivity testing of *Eimeria* oocysts recovered from litter and in the evaluation of live oocyst vaccines.

RESUMEN. Observaciones sobre la patología macroscópica de la infección de *Eimeria praecox* en pollos.

Las infecciones por desafío con 10^3, 5×10^4, 10^5, ó 5×10^5 ooquistes esporulados de *Eimeria praecox* causaron una moderada pero significativa reducción en la ganancia de peso con todas las dosis infecciosas. Se observó una reducción sustancial de los carotenoides en plasma y un aumento moderado, pero significativo en los $NO_2^- + NO_3^-$ en el plasma, pero solamente con las dos dosis más altas, dichos niveles fueron determinados en el día seis post-desafío. La revisión diaria de los pollos después del desafío con 5×10^4 ooquistes reveló una respuesta inflamatoria en el duodeno, y en el yeyuno, que comenzó al primer día después del desafío y que estuvo asociada con un aumento significativo en los niveles de $NO_2^- + NO_3^-$, que alcanzaron su punto máximo en el día cuarto después del desafío. Se observó una hiperplasia moderada y uniforme del intestino delgado y una depresión significativa de los carotenoides en plasma que se observó en los días 4 a 6 después del desafío. Los $NO_2^- + NO_3^-$ plasmáticos decrecieron hasta niveles control por el día seis después del desafío. Todas las infecciones estuvieron acompañadas por la producción de un exudado mucoide en el duodeno y en el yeyuno, que se convirtió en espeso y opaco por el cuarto día después del desafío y con la tendencia a enmascarar zonas con inflamación leve. Estas observaciones indican que la respuesta aguda del huésped contra la infección primaria con *E. praecox* es diferente y ocurre antes que la respuesta a las infecciones experimentales con otras especies de *Eimeria* spp., tales como *Eimeria acervulina*, *Eimeria maxima*, o *Eimeria tenella*. Estos factores deben ser considerados en las observaciones de la patología derivada de las co-infecciones de *E. praecox* con otras especies de *Eimeria*, especialmente en las pruebas de sensibilidad a las drogas, recuperación de ooquistes de *Eimeria* de la cama y en la evaluación de vacunas con ooquistes vivos.

Key words: *Eimeria praecox*, chickens, carotenoids, coccidiosis, inflammation, nitric oxide

Abbreviations: PC = postchallenge; PCR = polymerase chain reaction; USDA/ARS = U.S. Department of Agriculture/Agricultural Research Service

Although *Eimeria praecox* has been found worldwide, it is considered one of the less pathogenic species of coccidia that infect chickens, eliciting little or no pathology when given at experimental doses less than 10^4 oocysts (12,14,20). At higher doses (greater than 10^5 oocysts) its presence can have significant impact on production parameters such as weight gain and feed conversion (12,15,23,24,25). The *E. praecox* life cycle has been well studied (10,12,14,20,22), including observations on its fecundity (21,24). Although there are descriptions of gut pathology associated with high levels of infection (7,14,15,24,25), no pathognomic lesions have been reported, and there is no standard method for scoring this pathology equivalent to that described for other *Eimeria* species by Johnson and Reid (9). The purpose of the present study was to categorize the pathologic effects of *E. praecox* infection to better define its interactions with other *Eimeria* species. Several experiments were undertaken to augment information on the nature and timing of the host response to this parasite.

MATERIALS AND METHODS

Parasites. The strain of *E. praecox* (North Carolina 2) used in these studies is a field isolate originating from a broiler production facility in North Carolina. Individual *E. praecox* oocysts were isolated from feces containing *E. praecox* as indicated by morphology and confirmed by species-specific polymerase chain reaction (PCR) (8). Inocula containing 10–20 isolated *E. praecox* oocysts were given *per os* to susceptible chickens. Oocysts passed in feces between 83 and 91 hr were collected, sporulated, cleaned, and passaged three additional times with collections at about 83 hr. PCR, using primers specific for each of seven *Eimeria*

[A]Corresponding author. E-mail: mark.jenkins@ars.usda.gov

species (8), confirmed the purity of the *E. praecox* isolate (data not shown). Sporulated oocysts were stored in potassium dichromate at 4 C. Just prior to infection, the dichromate was removed by repeated washing (4–5 times) of oocysts with water followed by centrifugation, and oocyst suspensions were diluted in tap water to desired concentrations.

Chickens and housing. Cockerels (Sexsals-White Rock × Rhode Island Red) were obtained as day-old birds from Moyers Hatchery, Quakertown, PA, and were housed in a coccidia-free building until 3 wk of age. The chickens were then transferred to suspended cages, four to five birds per cage, in a building maintained at 28 C. Standard broiler starter ration (24% protein) and fresh water were provided *ad libitum* to all chickens. All animals were handled in accordance with protocols approved by the U.S. Department of Agriculture/Agricultural Research Service (USDA/ARS) Beltsville Animal Care and Use Committee.

Experiment 1. The objective of this experiment was to study the effect of various *E. praecox* oocyst challenge doses on several disease parameters and to characterize the pathology caused by a 5×10^5 oocyst dose. Chickens were randomized by weight (6) and placed into 5 groups of 8 birds/group. Groups of chickens differed by the dose of sporulated *E. praecox* oocysts administered: 0, 1×10^3, 5×10^4, 1×10^5, or 5×10^5 oocysts/bird given *per os*. At day 6 PC, chickens in all groups were bled by wing-vein puncture, killed by cervical dislocation, and scored for gross pathology of the duodenum and upper jejunum. In addition, weight gain during the infection period, plasma carotenoids, and $NO_2^- + NO_3^-$ levels were determined using standard procedures (3). Fecal oocysts were collected every 24 hr from all infected groups at 96, 120, and 144 hr PC. In order to characterize the pathology caused by a high dose of *E. praecox*, chickens in a separate group were inoculated with 5×10^5 sporulated oocysts. Individual chickens from this group were necropsied on successive days after challenge, and the upper intestine examined for gross pathology, which included observation of changes in the appearance of the mucosa in the duodenum and upper ileum.

Experiment 2. The objective of this experiment was to observe pathologic changes in the upper intestine of chickens infected with 5×10^4 *E. praecox* oocysts on days 1–6 PC. Chickens were randomized according to weight (8) and placed into 8 groups of 10 birds/group. Seven groups were infected with 5×10^4 oocysts per chicken. One of the *E. praecox*–infected groups was used for determination of oocyst production at 24 hr intervals from days 4–7 PC, while one group served as an uninfected control. On each of days 1–6, chickens in one infected group were weighed, bled by wing-vein puncture, killed by cervical dislocation, and necropsied for scoring gross pathology of the upper intestine. Weight gains, plasma carotenoids, and $NO_2^- + NO_3^-$ levels were determined using standard procedures (3). The length of duodenum, jejunum, ileum, and entire small intestine of infected chickens were measured on days 1–6 PC and compared to those of control chickens at day 6 PI.

Pathologic examination. Chickens were necropsied, and the upper intestine from the duodenum to the ileum was examined. On days 1–6 PC, gross lesions were scored on a scale of 0–4 based on the extent of mucoid exudate, appearance of inflammation, and paleness of the mucosa.

Statistics. Mean weight gain, mucoid exudate scores, inflammatory response, carotenoids, $NO_2^- + NO_3^-$ levels, and oocyst output were compared between treatment groups using Duncan's MultiRange Analysis (SAS, Cary, NC). In Experiment 1, oocyst output was compared between groups on each collection day using Duncan's MultiRange Analysis (SAS). Statistical significance between groups was considered if $P \leq 0.05$.

RESULTS

Experiment 1. Aside from the 10^5 dose level, all groups infected with *E. praecox* exhibited a significant ($P < 0.05$) reduction in mean weight gain compared to the uninfected control group (Fig. 1A). No significant differences ($P > 0.05$) were observed in weight gain between groups infected with different *E. praecox* doses (Fig. 1A). Also, no significant differences in gross pathology scores were observed among infected groups (Fig. 1B). Plasma carotenoids exhibited a significant reduction ($P < 0.05$) at all levels of infection, with the greatest reduction occurring in groups infected with 10^5 or 5×10^5 *E. praecox* oocysts (Fig. 1C). Plasma $NO_2^- + NO_3^-$ levels were significantly increased only in groups infected with 10^5 or 5×10^5 oocysts (Fig. 1D).

Oocyst shedding was dependent on the initial challenge dose and the time postinfection. For instance, chickens infected with 10^3 oocysts *E. praecox* shed the highest number of oocysts between 72 and 96 hr postinoculation, which was significantly greater ($P < 0.05$) than oocyst output in groups infected with greater numbers of oocysts (Fig. 2). At this time interval (72–96 hr), there appeared to be an inverse relationship between inoculation dose and oocyst output (Fig. 2). During the second 24 hr period (96–120 hr), oocyst output was highest in chickens infected with 10^3 oocysts and was significantly higher ($P < 0.05$) than the output arising from the other dose levels (Fig. 2). During the third 24 hr period (120–144 hr), oocyst output from chickens infected with 10^3 and 5×10^4 *E. praecox* oocysts declined, and oocyst output from chickens infected with 5×10^5 oocysts was highest (Fig. 2). At this time interval (120–144 hr), there was a direct relationship between *E. praecox* infective dose and oocyst output (Fig. 2).

Pathologic manifestations of infection with 5×10^5 oocysts were observed in the duodenum and upper jejunum. Consistent and reddened areas of apparent inflammation, a coagulated appearance of intestinal contents, copious cloudy mucoid secretions, and a paleness of the mucosal surface were noted, without discrete lesions. In particular, on day 1 PC the duodenal and jejunal mucosa acquired a slightly pinkish cast with increased mucus secretion. On day 2 PC, there was pronounced increase in redness of the mucosa at the bend of the duodenal loop, and in the area on either side of the bile and pancreatic ducts. On day 3 PC, the mucosa in areas on either side of the duodenum appeared thickened and inflamed as did the mucosa in the upper jejunum. On day 4 PC, although general redness appeared to subside, definite areas of petechiae were found in the subvillar layers after scraping off the top mucosa (Fig. 3). On days 5–6, mucosal surfaces of the duodenum were covered with mucus with sloughed mucosal cells mixed with ingested feed. Occasional reddish focal spots in both the duodenum and upper ileum were observed.

Experiment 2. Chickens challenged with 5×10^4 *E. praecox* oocysts showed constant weight gain from days 1–6 PC. On day 6 PC, mean weight gain of challenged chicks was lower, but not significantly different ($P > 0.05$) from weight gain in uninfected controls (Fig. 4A). Infection with *E. praecox* caused significant reduction ($P < 0.05$) of plasma carotenoids from days 4–6 PC (Fig. 4B). Aside from day 6 PC, plasma $NO_2^- + NO_3^-$ exhibited a significant increase ($P < 0.05$) at all time points compared to uninfected controls (Fig. 4C).

On days 4–6 PC, the mean length of the small intestine of infected chickens increased significantly ($P < 0.05$) compared to controls, exhibiting the greatest increase on day 5 PC (Fig. 4D). This increase occurred fairly uniformly throughout the intestine (duodenum + 120%, jejunum + 125%, ileum + 129%). Infection with 5×10^5 oocysts produced red, inflamed mucosa in specific areas of the duodenum and upper jejunum, particularly at the bottom of the duodenal loop, as early as day 1 PC (Fig. 5). This inflammation was greatest on days 2–3 PC. Small inflamed areas in the duodenum and jejunum could still be found through day 6 after the exudates were removed from the mucosal surface. Inflammation

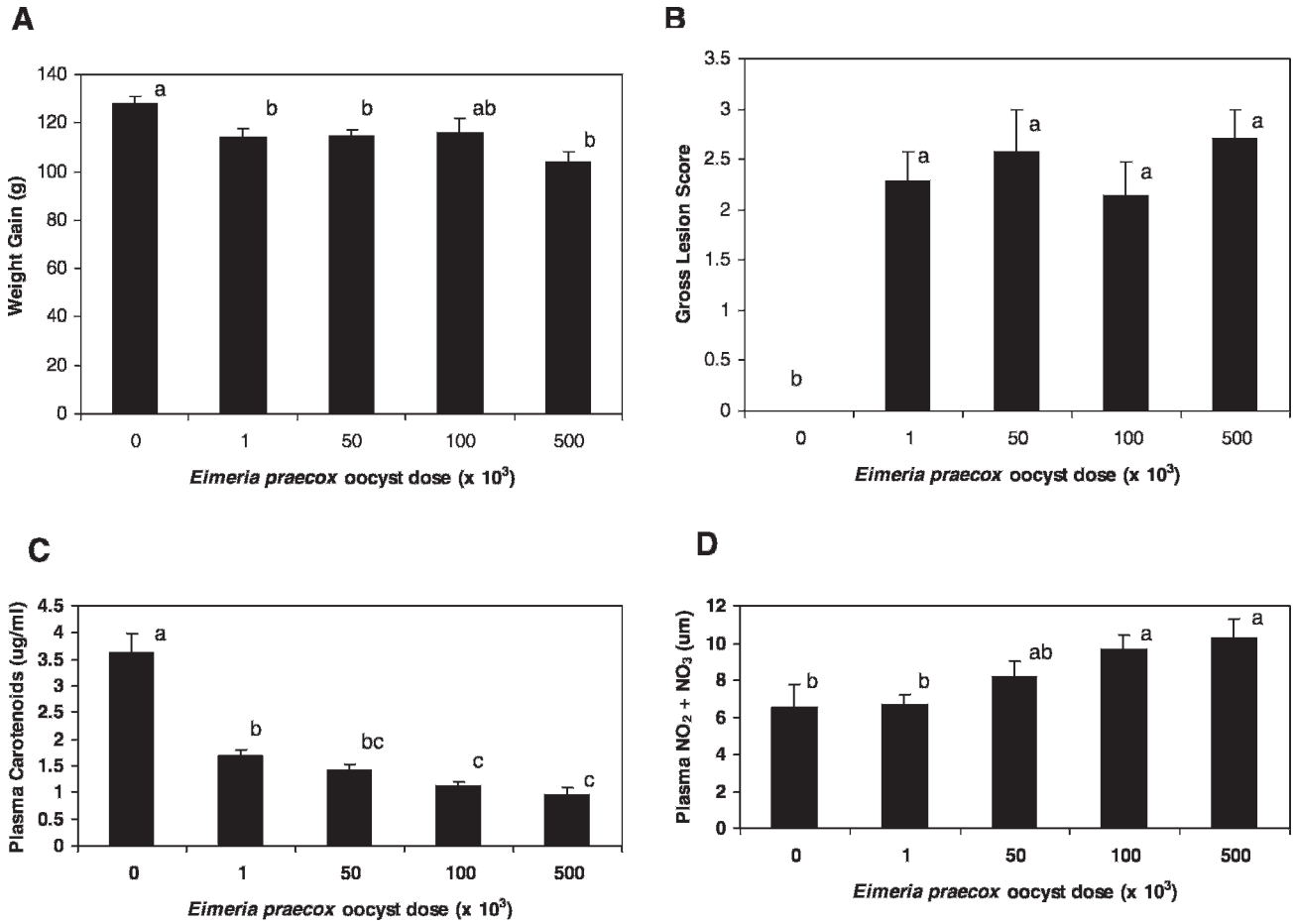

Fig. 1. Effect of increasing doses of *E. praecox* measured at day 6 PC on (A) weight gain, (B) gross intestinal pathology using an arbitrary scale from 0 to +4, (C) plasma carotenoids, and (D) plasma $NO_2^- + NO_3^-$ levels. Within a chart, values of bars sharing a letter are not significantly different from one another ($P > 0.05$).

of the combined duodenal and jejunal areas was evident on days 1–6 PC and were highest on day 2 PC (Fig. 6A). Mucoid exudates, which were evident from day 1 PC, tended to become heavier and more pervasive over time, being maximal by day 4 PC, which made differential scoring difficult, especially by days 5 and 6 (Fig. 6B).

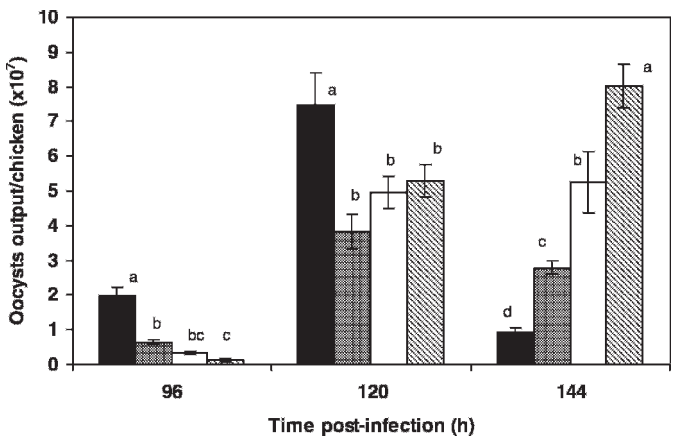

Fig. 2. Relationship of oocyst output to increasing infective doses of *E. praecox* oocysts challenge doses: Solid bar, 1×10^3 oocysts; gray bar, 5×10^4 oocysts; open bar, 10^5 oocysts; hatched bar, 5×10^5oocysts. At each time point, values of bars sharing a letter are not significantly different from one another ($P > 0.05$).

The exudate displayed a definite milky appearance by day 5 PC. The Peyer patch in the distal portion of the duodenum became noticeable by day 5 PC. Subvillar petechiae could also be found in the mid-anterior portion of the duodenum (Fig. 3), a finding consistent with observations reported by others (20). Mucosal paleness was quite evident by day 4 PC (Fig. 6C). Oocysts were detectable by day 4, peaked at day 5, and decreased by day 7 PC (Fig. 6D).

DISCUSSION

Eimeria praecox is reported to develop rapidly after mucosal penetration, and to carry out its entire life cycle within the epithelial cells of the superficial villi of the upper small intestine (13). It is thought not to develop in other types of cells, although sporozoites have been found in intraepithelial lymphocytes (5). The life cycle of *E. praecox* has been described in detail by a number of researchers (10,12,14,20,22), and yet its pathologic effects remain controversial. Some researchers find weight gain effects only at high infection doses, while others find significant weight gain depression at lower challenge doses (7,12,15,23,25). In the present study, it was demonstrated that *E. praecox* had a significant negative impact on weight gain over a wide range of infective doses (10^3–5×10^5 oocysts/chicken). It is possible that discrepancies in the literature are due to a number of factors, including genetics of the avian host and the particular *E. praecox* strain used in each study.

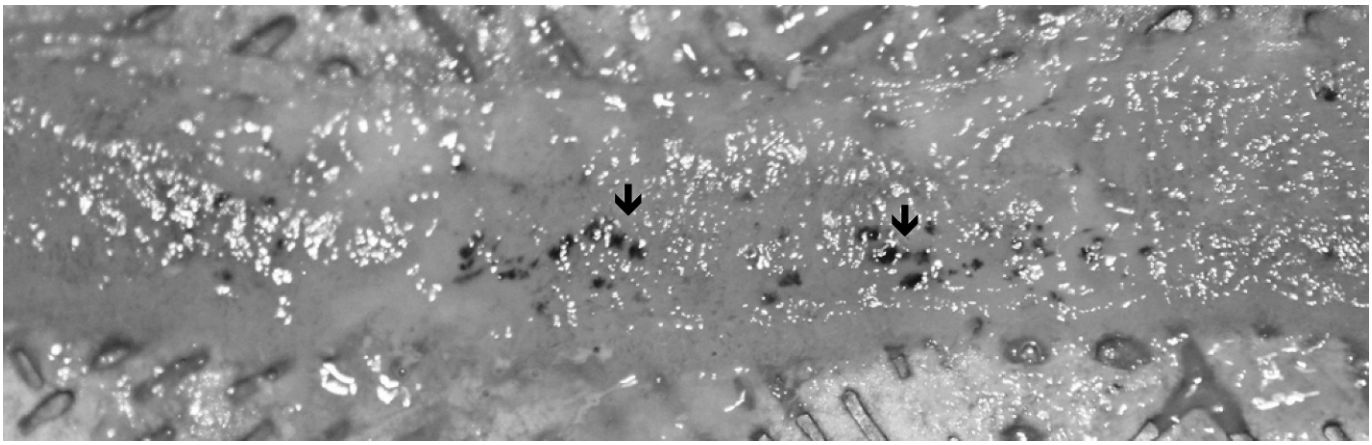

Fig. 3. Petechiae (arrows) observed in duodenum at day 4 PC after gently scraping away mucoid secretions and upper villar layer.

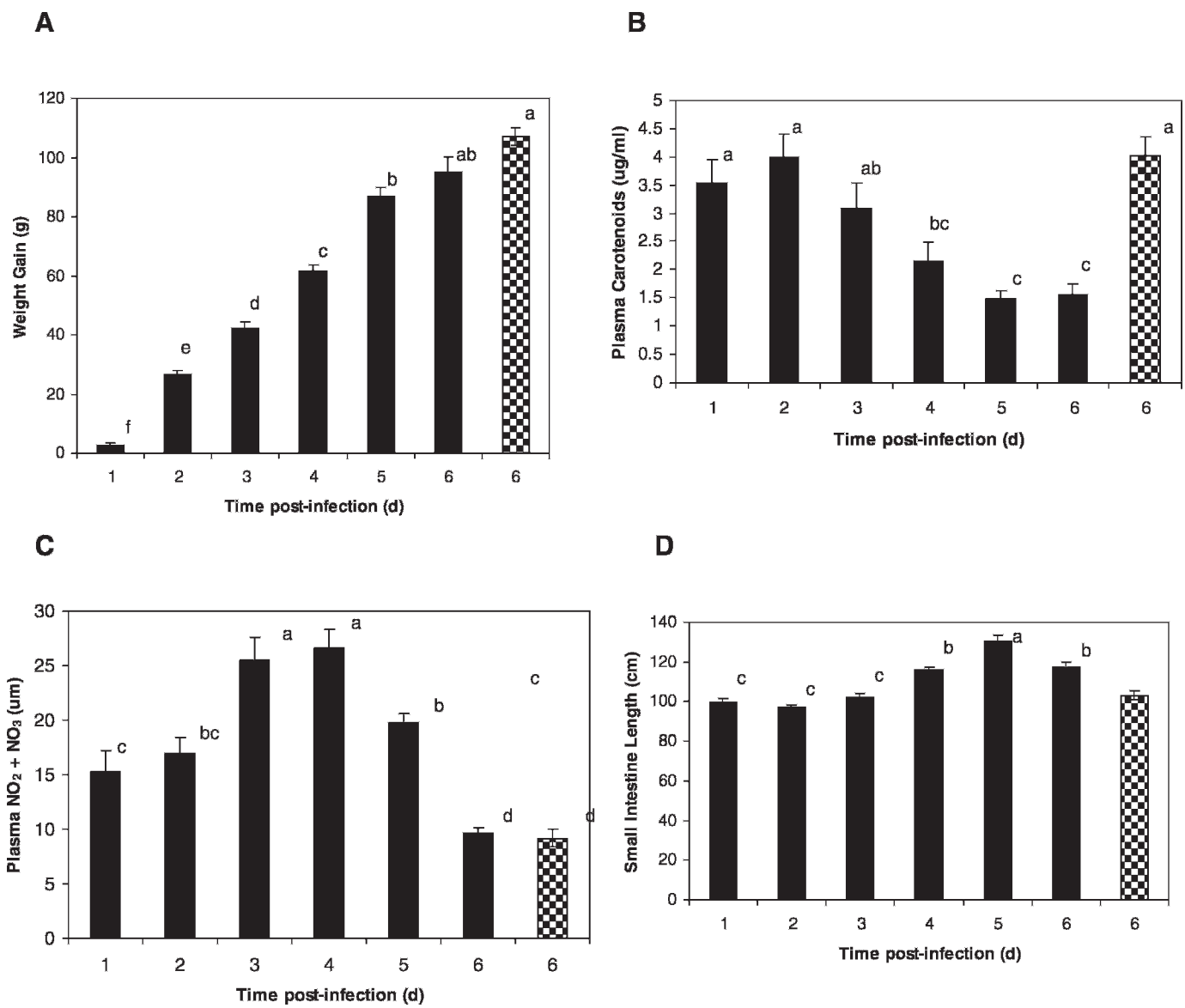

Fig. 4. Effect of challenge infection with 5×10^4 *E. praecox* oocysts measured (A) daily through day 6 PC on (A) daily weight gain, (B) plasma carotenoids, (C) plasma levels of $NO_2^- + NO_3^-$, (D) length of small intestine. The black bars denote the challenged groups, and the checkered bars denote the control group. Within a chart, values of bars sharing a letter are not significantly different from one another ($P > 0.05$).

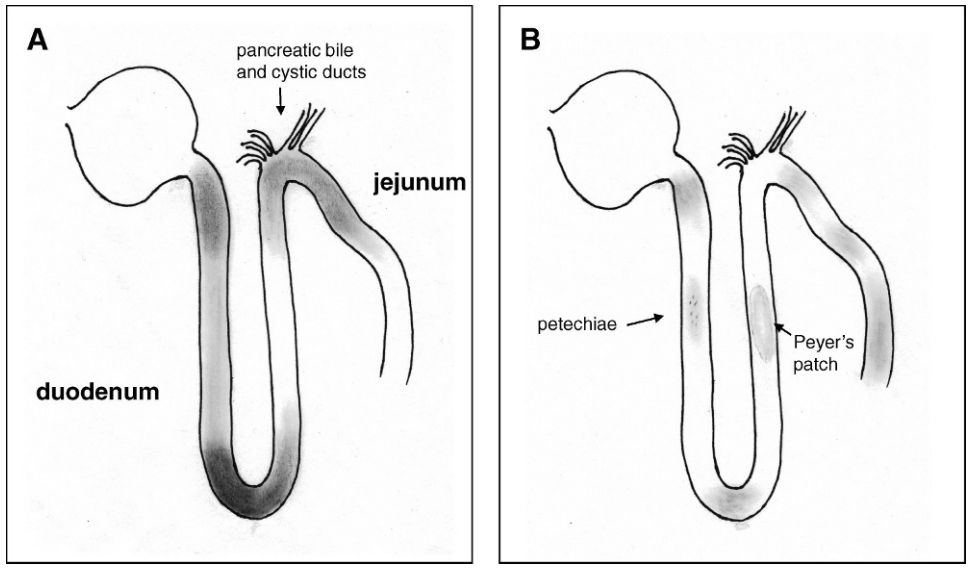

Fig. 5. Diagrams of inflamed areas of the chicken duodenum observed at different times PC with 5×10^4 *Eimiera praecox* oocysts. (A) Days 1–3 PC, (B) days 4–6 PC.

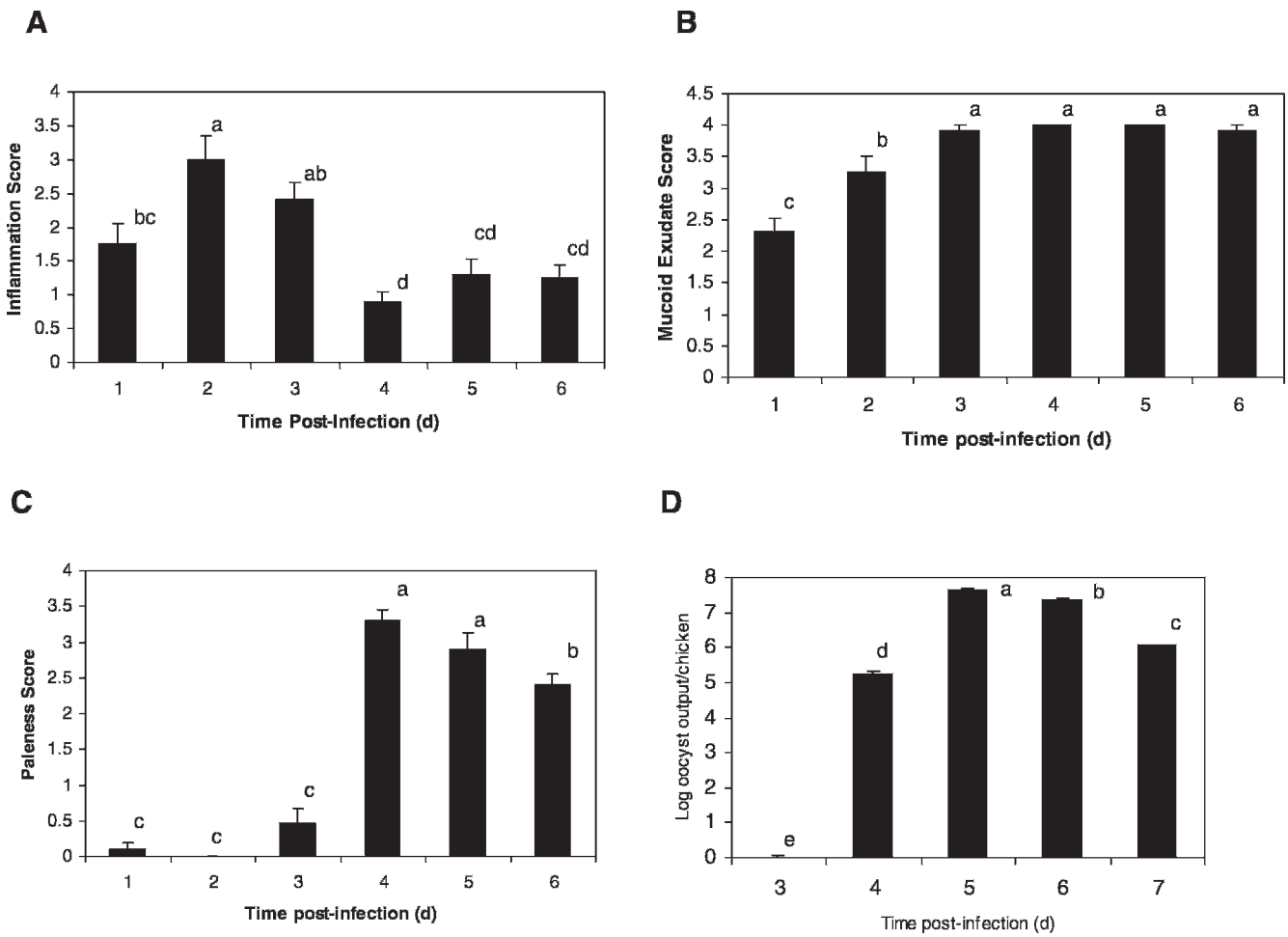

Fig. 6. Daily estimates, using an arbitrary scale of 0 through +4, of pathology associated with and oocyst output after a challenge dose of 5×10^4 *Eimeria praecox* oocysts. (A) Relative estimation of inflammation, (B) relative estimation of mucoid exudate, (C) relative estimation of mucosal paleness, (D) oocysts shed per chicken during 24-hr fecal collections made on day 4 through day 7 PC. Within a chart, values of bars sharing a letter are not significantly different from one another ($P > 0.05$).

No pathognomic lesions, such as seen for the more pathogenic species, have been reported for *E. praecox* infections. Pathology has been described only in general terms and includes mention of enteritis, diarrhea (23), increased vascular permeability in the gut (14,17), and production of mucoid exudates, the extent of which appear to be dose-related (15). In our study, pathology was judged in large part on the extent of mucoid exudate present and paleness, but no effect of increasing doses on either mucoid exudate or paleness were noted at day 6 PC. An interesting phenomenon was that the greatest amount of exudate appeared on day 3 PC, while paleness (the most subjective parameter) was most evident on day 4 PC.

In chickens infected with 5×10^5 *E. praecox* oocysts (Experiment 1), early inflammation of the duodenum and jejunum was observed. Moreover, a lower challenge dose (5×10^4 *E. praecox* oocysts; Experiment 2) led to consistent inflammation of the duodenum and jejunum. The timing of this inflammatory response coincides with the rapid series of four asexual reproductive steps and increased vascular permeability (14,17) within 3 days after inoculation, a phenomenon similar to that reported by others (7,14,15,20)

Intestinal lengths were measured during infection (Experiment 2) to determine whether *E. praecox* was capable of causing hyperplasia of the gut similar to that seen with *E. acervulina* infections (1), which is thought to be associated with compensatory nutrient absorption (19). However, only a modest uniform increase that coincided with oocyst shedding was observed, suggesting that nutrient uptake is not severely affected by *E. praecox* infection. This finding may explain why *E. praecox* has a lesser effect on weight gain compared to other *Eimeria* species, such as *E. acervulina* and *E. maxima*.

Depression of plasma carotenoids associated with infections from pathogenic species that infect the upper small intestine, such as *E. acervulina* and *E. maxima*, have usually been a sign of nutrient malabsorption associated with structural damage to the absorptive mucosa by the parasites (18). Others have reported that mild infection with *E. praecox* (10^3 oocysts) had no significant effect on carotenoid absorption (16). However, in both Experiments 1 and 2, significant decreases in plasma carotenoids occurred, even at a dose as low as 10^3 oocysts. It seems illogical to conclude that these decreases result from nutrient malabsorption, because effects on weight gain, although significant, were slight (8.5–16.3%). One possibility is that increased vascular permeability as reported by others may adversely affect dietary carotenoid absorption. Another possibility is that carotenoids could be destroyed through actions of reactive free radicals associated with immune responses (2). In Experiment 2 a significant reduction in carotenoids was observed after the reported period of asexual replication of the parasite.

In this laboratory, plasma levels of carotenoids and $NO_2^- + NO_3^-$ have been used routinely as biomarkers to gauge severity of coccidia infections. Primary infections with more pathogenic coccidia such as *E. acervulina*, *E. maxima*, and *E. tenella* produce strong $NO_2^- + NO_3^-$ responses (3), an index of nitric oxide produced during a cellular immune response. There is a reciprocal relationship between plasma carotenoids and $NO_2^- + NO_3^-$ that is useful in scoring pathology and protective actions of anticoccidial treatments (3). It therefore seemed unusual to find, in response to a range of *E. praecox* challenge doses, that, although plasma carotenoids were depressed throughout, plasma $NO_2^- + NO_3^-$ levels were elevated only slightly and only at high challenge doses when measured at 6 days PC. However, results of Experiment 2 show that infection with *E. praecox* does elicit a $NO_2^- + NO_3^-$ response as early as day 1 PC, and that this coincides with appearance of inflamed areas in the duodenum and jejunum. This $NO_2^- + NO_3^-$ response may represent a cellular immune response of the host to penetration of epithelial cells by zoite stages (17) and intracellular development of schizonts and may be related to the increased vascular permeability observed by others (14,17).

The inverse relationship observed at 96 hr PC between oocyst production and infective dose, and direct relationship between these two parameters at 144 hr PC, possibly reflects a ''crowding effect'' phenomenon (4,11,24). Crowding is thought to occur at high challenge doses that lead to competition for available noninfected epithelial cells of the intestinal villus, and thus fewer oocysts per oocyst inoculated are produced (24). For *E. praecox*, the crowding threshold has been estimated to be 16 oocysts (24), which is considerably less than the lowest challenge dose (10^3 oocysts) used in the present study. Although there were differences in oocysts production over time between different *E. praecox* challenge doses, total oocyst production over the 72 hr collection period showed no appreciable differences among infection levels ($4.2 \times 10^9 \pm 0.9 \times 10^9$). It is possible that *E. praecox* developmental stages invade areas of the gut proximal to the upper–mid-intestine to complete subsequent asexual development and zygote formation.

In summary, the pathologic changes caused by *E. praecox* were characterized and, dissimilar to other more pathogenic *Eimeria* species, appear to occur rapidly following challenge infection. Although pathologic effects of most *Eimeria* infecting chickens (e.g., *E. acervulina*, *E. maxima*, and *E. tenella*) are usually observed at days 5–6 PC, pathology associated with *E. praecox* infection should be scored on days 3 and 4 PC.

REFERENCES

1. Allen, P. C. Physiological responses of chicken gut tissue to infection with *Eimeria acervulina*. Avian Dis. 28:868–876. 1984.

2. Allen, P. C. Production of free radical species during *Eimeria maxima* infections in chickens. Poult. Sci. 76:814–821. 1997.

3. Allen, P. C., H. D. Danforth, and B. Vinyard. Development of a protective index to rank effectiveness of multiple treatments within an experiment: application to a cross protection study of several strains of *E. maxima* and a live vaccine. Avian Dis. 48:370–375. 2004.

4. Brackett, S., and A. Bliznick. The reproductive potential of five species of coccidia of the chicken as demonstrated by oocyst production. J. Parasitol. 38:133–139. 1952.

5. Fernando, M. A., M. E. Rose, and B. J. Millard. *Eimeria* sp. of domestic fowl: the migration of sporozoites intra- and extra-enterically. J. Parasitol. 73:561–567. 1987.

6. Gardiner, J. L., and E. E. Wehr. Selecting experimental groups of chickens by weight. Proc. Helm. Soc. Wash. 17:25–26.1950.

7. Gore, T. C., and P. L. Long. The biology and pathogenicity of a recent field isolate of *Eimeria praecox* Johnson 1930. J. Protozol. 29:82–85. 1982.

8. Jenkins, M. C., K. Miska, and S. Klopp. Improved polymerase chain reaction technique for determining the species composition of *Eimeria* in poultry litter. Avian Dis. 50:632–635. 2006.

9. Johnson, J. K., and W. M. Reid. Anticoccidial drugs: lesion scoring techniques in battery and floor pen experiments with chickens. Exp. Parasitol. 28:30–36.1974.

10. Johnson, W. T. Director's biennial report: 1928–1930. Corvallis: Oregon Agricultural Experiment Station. 1930.

11. Johnston, W. T., M. W. Shirley, A. L. Smith, and M. B. Gravenor. Modelling host cell availability and the crowding effect in *Eimeria* infections. Int. J. Parasitol. 31:1070–1081. 2001.

12. Jorgensen, W. K., N. P. Stewart, P. J. Jeston, J. B. Molloy, G. W. Blight, and R. J. Dalgliesh. Isolation and pathogenicity of Australian strains of *Eimeria praecox* and *Eimeria mitis*. Aust. Vet. J. 75:592–595. 1997.

13. Lee, D. L., and B. J. Millard. Fine structure of the schizonts of *Eimeria praecox*. Int. J. Parasitol. 1:37–41. 1971.

14. Long, P. L. Studies on *Eimeria praecox* Johnson 1930 in the chicken. Parasitology 57:351–361.1967.

15. Long, P. L. The pathogenic effects of *Eimeria praecox* and *E. acervulina* in the chicken. Parasitology 58:691–700. 1968.

16. Marusich, W. L., E. F. Ogrinz, E. Schildknecht, P. R. Brown, and M. Mitrovic. The effect of subclinical infections with *Eimeria praecox* and *Eimeria tenella* on pigmentation and vitamin A absorption in broilers. Br. Poult. Sci. 14:541–546. 1973.

17. Rose, M. E., and P. L. Long. Immunity to coccidiosis: Gut permeability changes in response to sporozoite invasion. Experientia 25:183–184. 1969.

18. Ruff, M. D., and H. L. Fuller. Some mechanisms of reduction of carotenoid levels in chickens infected with *Eimeria acervulina* or *E. tenella*. J. Nutr. 105:1447–1456. 1975.

19. Ruff, M. D., and G. C. Wilkins. Total intestinal absorption of glucose and L-methionine in broilers infected with *Eimeria acervulina*, *E. mivati*, *E. maxima* or *E. brunetti*. Parasitology 80:555–569. 1980.

20. Salisch, H. A study of the life cycle of *Eimeria praecox*, Johnson 1930. Zentralbl. Vet. B 37:363–368. 1990.

21. Shirley, M. W., V. McDonald, H. Chapman, and B. Millard. *Eimeria praecox*: selection and characteristics of precocious lines. Avian Pathol. 13:669–682. 1984.

22. Tyzzer, E. E., H. Theiler, and E. J. Jones. Coccidiosis in gallinaceous birds. II. A comparative study of species of Eimeria in the chicken. Am. J. Hyg. 15:319–393. 1932.

23. Williams, R. B. Epidemiological aspects of the use of live anticoccidial vaccines for chickens. Int. J. Parasitol. 28:1089–1098. 1998.

24. Williams, R. B. Quantification of the crowding effect during infections with seven *Eimeria* species of the domestic fowl: its importance for experimental designs and the production of oocyst stocks. Int. J. Parasitol. 31:1056–1069. 2001.

25. Williams, R. B., R. N. Marshall, M. Pagés, M. Dardi, and E. del Cacho. Pathogenesis of *Eimeria praecox* in chickens: virulence of field strains compared with laboratory strains of *E. praecox* and *Eimeria acervulina*. Avian Pathol. 38:359–366. 2009.

ACKNOWLEDGMENTS

The authors acknowledge the excellent technical assistance of USDA employee Eliseo Miramontes.